THE ORIGIN OF NATURAL IMMUNITY TOWARDS THE PUTREFACTIVE BACTERIA.

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Immunity towards the putrefactive bacteria, that normally inhabit the intestine, is but a part, although an important one, of the whole subject of immunity. It is, as we shall see, intimately related to natural immunity towards stray pathogenic bacteria that may obtain access to the tissues of an animal. This form of immunity is the first line of defence of the animal, the second being the production of anti-toxines in response to the presence, action or effect of micro-organisms that have obtained a foothold in the tissues. Individuals, races and genera of animals exhibit differences in their susceptibility towards bacterial invasion, but this is a question subsidiary to that of a general immunity such as all animals possess towards the bacteria that normally inhabit the intestinal tract. It is towards the elucidation of this general immunity that I bring forward certain views which appear to me to be feasible and worthy of consideration.

There is a tendency among writers to consider immunity against bacteria as being in some way bound up with enzyme action, and perhaps Pfeiffer may be cited as holding the most advanced views in this direction. But beyond expressing the idea that the action is enzymic, little is brought forward to explain how the enzymes may act, and probable analogies are rarely quoted. Immunity against toxines appears to be of a definite chemical nature in opposition to the indefinite action that is generally associated with the name of enzyme. The combination between the anti-toxine and toxine is like that which occurs when an acid unites with a base. Many writers have emphasised this, but probably none so clearly as Madsen and Arrhenius, who, while showing that the union is like that of a strong base with a weak acid, such as boric, have cleared the path of certain of Ehrlich's degraded toxines that served only to obscure the view.

Associated with immunity there is a phenomenon known as agglutination, which, like toxine immunity, obeys the laws of chemical combination. The immobilisation of the bacteria by an agglutinating serum is caused by the formation of a precipitate between the bacterial product and agglutinine under the influence of the residual affinity of a flocculating salt or other substance. Peculiarities in the flocculating action have lately been noted by Dreyer,* who, while comparing some of the actions with those exhibited by chemical substances, such as the combination of gum mastic and ferric chloride, at another place speaks of a destruction of agglutinine by caustic alkalies, which is restored by acids. This is an example of the tendency shown, even among those investigators who incline to the chemical nature of the phenomenon of agglutination, to regard it sometimes in a chemical light and sometimes as a mysterious something with laws of its own. Flocculation has not been deeply studied, especially with regard to the precipitation of the weak organic acids and bases, but when it shall have been, there can be no doubt that parallels will be found to show that agglutination and precipitation are one and the same thing.

But in returning to the origin of immunity, it will be necessary to consider the possibility of bacteria passing from the intestine into the tissues. Schott,† in reviewing the literature of the subject up to 1901, concluded that we were not justified in assuming that pathogenic or non-pathogenic bacteria could pass through the wall of the uninjured alimentary canal. Rogozinski‡ found that the mesenteric glands always contained bacteria, while the chyle, liver and spleen were free. Faltin§ found that bacteria

* Brit. Med. Journ. Sept. 10, 1904, No. 2280, 564.
† Cent. f. Bakt. 1, xxix. 239.
‡ Cent. f. Bakt. 1, Ref. xxxiv. 323.
§ Cent. f. Bakt. 1, Ref. xxxi. 544.

may pass to the kidney, and thence get to the bladder. Klimenko,* after reviewing his own work and that of others, concluded that micro-organisms could not pass through the unhurt intestinal wall of thoroughly healthy animals, but that the latter were very seldom to be met. The smallest pathological injury of the whole animal organism, or an insignificant mechanical injury of the intestinal mucosa, is enough to render possible the passage of bacteria from the intestine. On this account the transmigration of micro-organisms is relatively frequent. From the frequency with which bacteria were met with in the mesenteric lymph glands, and not in the other internal organs, he considered that it is probable that the animal possesses in these a protective apparatus against bacterial invasion.

Nikolsky† mixed anthrax spores with the food of various animals and found that they germinated in the contents of the intestine in spite of the antagonism of the intestinal bacteria and, as a rule, penetrated the mucous membrane, and so obtained access to the lymph vessels and thence to the blood. Metchnikoff mentions some unpublished work of Mitchel, who mixed anthrax spores with the food of one set of guinea-pigs, and anthrax spores and glass with the food of another. He always obtained fatal results in the latter set of animals, while in the former the results were not always so pronounced. He also quotes the experiments of Porcher and Desoubry, who showed that the chyle of the dog contained bacteria capable of growing in ordinary media that could pass into the general circulation and be recovered therefrom. These disappear soon after feeding, and in the practice of obtaining curative sera it is customary to bleed the horses or other animals fasting in order to obtain a serum free from microbes.

It is apparent from these abstracts that there is a considerable divergence of opinion among investigators as to whether or not bacteria traverse the uninjured intestinal wall, and perhaps there always will be, for reasons which I shall subsequently give.

* Zeit. f. Hyg. xlviii. 67.

† Ann. Inst. Past. xiv. 794.

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Before doing so, however, let me bring forward the subject of the production of enzymes.

Oppenheimer,* after discussing the production of enzymes, says :—"The mould-fungi produce no ferments so long as they are grown upon media from which they can directly supply their wants; but they immediately develop proteolytic enzymes when they are cultivated upon a culture medium containing albumin, diastase when they are supplied with starch, and so on." In the higher plants it is known that there are no enzymes in the resting seed, and that under suitable conditions of moisture and temperature the presence of food, capable of being digested, determines the formation of the digestive ferments. In the animal body it has been found that the digestive enzymes of the stomach are secreted in response to the presence and even to the sight of food; and furthermore, the nature of the food appears to cause the secretion of the appropriate digestive enzyme. Bayliss and Starling,† in discussing the alteration of the pancreatic enzymes according to the nature of the food, quote the experiments of Pawlow and his pupils, which showed that a starchy food caused a rise in the amylolytic power, and a meat food a rise in the proteolytic power, and fats in the fat-splitting power. While doubting the accuracy of the conclusions concerning the proteolytic secretions, they considered that "there seems no reason to doubt the results obtained by these workers as regards the starch-digesting power of the juice."

This, however, applies to a case or cases in which the digestive apparatus has been acting for some time, and not to the original production of the ferments. I understand that rennin† is obtained from the stomachs of calves which have digested milk,

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* Ferments and their Actions, p.78.

† Proc. Roy. Soc. 1904, lxxiii. [494] 318.

* Rennin is not a digestive enzyme in the ordinary sense, although it undoubtedly is one, for does it not split up casein into paracasein and a soluble albuminoid or albumose? This partial digestion appears to be the function of rennin in the animal stomach. The faeces of children fed solely with cow's milk appear to consist chiefly of paracasein.

and that when the calf ceases to be carnivorous and becomes herbivorous, the secretion of rennin ceases. This may be so in practice, but Moro* found rennin in the mucous membrane of the stomachs of children soon after birth and before feeding. A dead-born child has none. The loss of weight of an animal during the first week or ten days after birth can probably be explained by a certain time being necessary to get the digestive system into working order in response to the stimulus given by the presence of food in the alimentary tract. As we shall see, lactase takes some time before it is secreted. Portier obtained lactase from ducks after they had been fed with lactose for 25 days, and Bayliss and Starling say that "the pancreas of new-born animals, for instance, is quite free from lactase, which, however, makes its appearance two or three days after birth as a result of the milk diet. . . . For the production of lactase in the pancreas, or its juice, it is therefore necessary that lactose should act on the intestinal mucous membrane for some time. The reaction is a slow one . . . ."

It is evident from these few examples that digestive enzymes are originally and subsequently formed or secreted both by plants and animals in response to the stimulation made by the presence of the nutrient capable of being acted upon. The presence of food nutrients in the alimentary tract brings forth from the cells of the tract or glands connected therewith the appropriate digestive enzymes.

If dead bacteria were fed, or if living bacteria were killed in a part of the canal, it will not be gainsaid that they would share the fate of the food nutrients. Metchnikoff,† in discussing the rôle of microbes in the intestine, mentions Klein, Schutz and Kohlbrugge as having found a bactericidal action occurring in the intestine; the latter investigators traced the action to the small intestine. He quotes the results of some unpublished experiments of Delezenne, which showed that neither the pan-

* Cent. f. Bakt. Orig. xxxvii. 485.

† Bull. l'Inst. Past. i. 217.

creatic nor the intestinal juices were by themselves bacteriolytic, although together they were.

In the intestine we have a bactericidal action, and the dead cells will naturally call into being appropriate digestive enzymes; and furthermore, the constituents of the bacteriolysed cells will, together with the digested constituents of the dead micro-organisms, be absorbed by the mucous membrane, in the cells of which, or possibly in some more remote organ, they may, if the products of digestion can stimulate the formation of digestive enzymes, give rise to what are known as immune bodies.

When an animal dies, micro-organisms swarm into the tissues from the alimentary tract, and soon there is an active decomposition of the whole organic structure of the corpse. Why does not this occur in the living animal? That the mucous membrane of the alimentary, and especially of the intestinal, tract is impervious to bacteria because the animal, and therefore the epithelial lining cells are endowed with life, is rather a feeble explanation, since it refers the question to the unquestionable. That the living tissues are normally protected admits of little doubt, but how are they so? It is known that the mother confers immunity upon the offspring, that is to say, she transfers a quantity of the immune bodies that she possesses. These are sufficient to render the young animal immune for some time. But when these are exhausted, as in time they must be, whence does it obtain its own supply? Are we justified in saying that the immunity bestowed upon the child by the mother is of a permanent and creative nature, that it persists throughout the life of the child, and is by it bestowed upon its descendants, generation after generation, through all time? No! Before the maternal allotment is exhausted the offspring must have manufactured, and be capable of producing on its own account, immune bodies of its own, among which are the bacteriolytic enzymes.

When weakened pathogenic bacteria are introduced into the tissues of an animal they are rapidly dissolved or digested, and at the same time they stimulate the cells to secrete the diverse immune bodies which confer an increased immunity upon the experimental animal, enabling it to attack and dissolve more virulent bacteria of the same kind. Thus immunity against one microbe is begotten, as it were, by the previous digestion in the tissues of the same micro-organism, and a multiplicity of immune substances can only be obtained by the previous lysis of a diversity of bacteria. In a young animal this must occur.

In writing upon the question of bovine and human tuberculosis, Behring appears to say :—" The freedom with which milk-bacteria find their way through the walls of the alimentary tract into the circulation, owing to the incomplete continuity of the epithelium and absence of active ferment secretion in young animals, makes the disposition to tuberculous infection entirely physiological and normal."* Without discussing the particular case of tuberculous infection, does it not seem possible that the non-continuity of the epithelium of the alimentary tract of young animals is the means by which an allwise Providence endeavours to accelerate the formation of protective bodies before the immunity bestowed upon them by the parents has become exhausted ?

We have seen that all investigators do not admit the passage of bacteria through the uninjured intestinal wall, for the reason that bacterial growths cannot always be obtained when portions of the organs or membrane are sown in nutritive fluids. The cause of this failure will be apparent if we assume that they are capable of passing through. In doing so, they will be attacked either there or in the more remote tissues, first, in the case of the young animal, by the maternal, then by the individual immune substances. During the lysis of these microbes the cells will be trained or stimulated to produce more immune bodies. It is also possible that the presence of bacteria in the intestinal tract causes the cells of the mucous membrane to secrete immunity enzymes which diffuse not only into the lumen, but also throughout the membrane. The diversity of bacteria produces a variety, and the continued solution of organisms will induce the formation of a quantity of immune substances. The quantity and the hetero-

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* Nature, June 6th, 1904, p.126.

genity of these produce the condition known as natural immunity. As the bacteria are constantly traversing the mucous membrane and being dissolved, the stock of immune bodies is being constantly replenished. With a perfectly sound and healthy animal there will be an abundance, so to speak, of immune bodies, and the bacteria will in consequence be dissolved soon after leaving the lumen of the intestine. They will not get so far as the organs which will be found to be sterile, so that investigators might conclude that bacteria given with the food do not leave the intestine. Should the animal not be perfectly healthy and the store of immune bodies low, the intestinal micro-organisms will probably travel further and be found in the glands and organs. The same thing would happen if the intestine were injured, for the numbers crossing the wall would be relatively enormous, and the small supply of immune bodies would be quickly used up. While positive results, obtained by sowing mucous membrane and lymphatic organs in nutritive media, are undoubted proofs of the passage of bacteria from the intestine, negative results simply show the absence of living bacteria. The sterility may be due to no bacteria having traversed the intestinal wall or having crossed, they have been destroyed during or after the passage by the immune bodies. Experimental work upon this subject will, therefore, always be conflicting.

It is thus unreasonable to expect to obtain information regarding the permeability of the intestine to putrefactive and saprophytic bacteria by an examination of the organs. With pathogenic bacteria the case is different. If they survive the passage through the acid juice of the stomach, they will traverse the intestinal walls and, multiplying in the tissues and organs, will produce their characteristic lesions, provided that they can get across the mucosa in sufficient numbers to absorb any trace of specific immune body.

I have regarded the solution or lysis of bacteria as a process of digestion, all the substances which are necessary to dissolve the cells being called the bacteriolytic immune bodies. Several substances are involved in the lysis of one cell. Until quite recently two were known—the immune body and the complement

—but now we have a third, the opsonin. It is, perhaps, on account of this complexity that bacteriologists have refrained from regarding digestion and lysis as being analogous in other than a half-hearted manner, although it has recently been shown that the secretion of digestive enzymes is anything but a simple process. Emmerich and Loew have certainly emphasised the tryptic nature of their pyocyanase, but they practically stand alone in so emphatically regarding a digestive enzyme as being a source of immunity. Metchnikoff,* after mentioning Delezenne's work upon the antiseptic action of the intestinal juice, says that it proves for the first time the great analogy that exists between the mechanism of intestinal digestion and the bactericidal and hæmolytic effect of the blood sera. Wright and Douglas† think it probable that the bacteriolytic, bactericidal and bacterio-opsonic effects are each in their degree manifestations of a digestive power exerted by the blood fluids upon bacteria.

When a substance is introduced or finds its way into the tissues or into the body cavity the animal endeavours to get rid of the intruder. If the substance is soluble and diffusible it may be eliminated by way of the kidneys. If not, it may be altered into other bodies that can be so eliminated. Ignoring the case of substances that are absolutely insoluble and indigestible, we are left with organic bodies that are digestible. To this class belong the bacteria. When they are digested they are called harmless, but when they are not digested and, multiplying, produce toxines that interfere with the health of the animal, they are pathogenic. The difference between the two is that the pathogenic cell encounters no enzyme capable of dissolving it. It is a stranger, and has not recently been within the animal. Had the animal been immunised either by the introduction of weakened cells or of blood serum from immune subjects, the pathogenic cell would not have been a stranger and would have been dissolved like a harmless microbe. Although the cells of the body respond to

* Bull. l'Inst. Past. i. 228.

† Proc. Roy. Soc. lxxii. 369.

the presence of a foreign cell (microbe) and secrete immunity bodies that dissolve the intruder, it must not be forgotten that the microbe will in turn secrete anti-bodies which will annul the action of the immunity bodies. Welch‡ pointed this out, but why did he stop at the response of the microbic cell? The body cells will respond to the microbic anti-body, and so the production of enzymes and anti-enzymes, toxines and anti-toxines, will go on until the microbe or the animal is overcome. The digestion of bacteria may take place in the body fluids or in the body cells and tissues, which, it must not be forgotten, are saturated with the same fluids and contain the same digestive enzymes, if the latter are diffusible. That some of them are diffusible is shown by the bacteriolytic power of the fluids. It is inconceivable that immunity bodies can be generated in the fluids themselves. The constituents of these fluids must be produced and be contained in and be excreted or secreted by the wandering and fixed body cells, the plasma of which will be saturated. Digestion will, therefore, be more rapid when the micro-organism is within the body cell, and especially is this the case if the enzyme is feebly diffusible or non-diffusible. In the event of a bacterium getting into the body fluids it will be attacked by the enzymes of that fluid, and by the nearest cells which will also respond to the stimulus and secrete more. The cells which come into action are and must be the most mobile ones, for they get nearer to the intruders than the others; distance must be of great moment in supplying the stimulus. It would be foolish to expect that the cells of the ear would respond to the presence of an organism in the toe. It is because of the mobility of the leucocytes that we have the idea that they are chiefly responsible for the production of the immune digestive enzymes.

The inclusion or swallowing of the microbe by the leucocyte is not, strictly speaking, an immunity phenomenon, for any amœboid cell will surround and enclose any digestible or slowly soluble

‡ Brit. Med. Journ. Oct. 11th, 1902, p.1109.

particle.* In the case of pathogenic bacteria that resist the leucocytes, there must be something in or on the cells, or given out by them, of a leucocyte-repelling (negative chemotaxic) nature. What this is we do not at present know, but it is likely that there is much the same physical relation between the leucocyte and the capsule of the pathogenic microbe as there is between water and fat, so that if the capsule is partly digested by a cytase or covered with a layer of some substance which will annul the repelling influence, the bacterium will be mechanically absorbed by the leucocyte.

It is possibly here that agglutination enters actively into the arena of immunity. We know that bacteria, *e.g.*, typhoid, which have been treated with the agglutinine contained in patient's serum, become immobilised and gather into clumps through the formation and flocculation of a precipitate upon the capsules. We also know that after a time the bacteria regain their motility. The simplest reason to account for the reassumption of mobility is that the precipitate is slowly dissolved. The solubility of the covering would enable the microbe to be absorbed by the leucocyte† in the same way that a covering of shellac would enable a fragment of glass to be seized by a drop of chloroform. When the bacterium is within the protoplasm of the phagocyte, there ought to be an increased production of immune bodies. The reason for the precipitation of an albuminoid by a specific precipitin is doubtless to be found in the fact that the albuminoid is non-diffusible and does not get within the phagocyte. But what does get in contact with the surface of the cell calls forth a precipitin which coagulates the non-diffusible and foreign albuminoid. The coagulated particles are then rapidly engulfed by the phagocytes and are digested with a greater rapidity than would otherwise have been the case.

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* The purely physical nature of the movements and swallowing powers of amœbæ are well described in Journ. App. Micros. v. 1597.

† The subject is under investigation.

In this paper I have endeavoured to show :—

(1) That there is a close analogy or identity between the production of bacteriolytic bodies and the digestion of food.

(2) That bacteria do traverse the intestinal wall, and that negative experimental results regarding the same are untrustworthy.

(3) That natural immunity, especially towards the bacteria that normally inhabit the intestinal tract, is occasioned and maintained by the comparatively few bacteria which, in crossing the intestinal wall and possibly gaining access to the body fluids and organs, stimulate the cells to produce immune bodies.

(4) That the agglutination of bacteria may play a much more active part in the production of immunity than is generally supposed.